

**U.S. DEPARTMENT OF COMMERCE
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Preparation of Research Reagents for 2 Dengue Strains

Yale Univ, New Haven, Conn Yale Arbovirus Research Unit

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16. Abstracts Four lots totalling 3,050 ml of dengue 2 mouse immune ascitic fluid; 5 lots totalling 3,365 ml of dengue 3 immune ascitic fluid; and two lots of 200 ml each of control ascitic fluids were prepared and delivered to the NIH. Plaque reduction neutralization tests indicated that most lots of dengue 2 fluids had homologous neutralization about 32-fold greater than heterologous. Dengue 3 fluids were less specific, with homologous titers 4-8 fold greater than heterologous. Seed virus reagents for dengue 2 and dengue 3 viruses had titers of 9.0 log LD ₅₀ /ml and 7.0 log LD ₅₀ /ml respectively. Control ascitic fluids did not neutralize dengue viruses.			
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Title: Preparation of Research Reagents for 2 Dengue Strains

Report Number: RR-F-2509-76

Author: Dr. Robert E. Shope

Performing Organisation: Yale Arbovirus Research Unit
Yale University School of Medicine
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New Haven, Connecticut 06520

Date of Preparation: April 9, 1976

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I. Objective

Seed virus reagents, immune and control ascitic fluids for dengue, type 2 and dengue, type 3 viruses were to be produced, the fluids to be tested for homologous and heterologous plaque reduction neutralization test (PRNT), complement-fixation (CF), and hemagglutination-inhibition (HI) titers and to be shown to be free of adventitious agents. The seed virus reagents (SVR) were to be tested for potency and purity.

II. Materials and Methods

- a. Viruses. Dengue type 2 (NGC) (passage level man 4, mouse 22) from ATCC and dengue type 3 (H87) p.21 was obtained from W. McD. Hammon.
- b. Mice. Specific pathogen free Charles River mice from the YARU colony were used..
- c. Master Seed Virus. After a single passage in baby mice, 50 x 1 ml of 20% master seed virus were prepared and stored at -70°C.
- d. Seed virus reagent. Master seed virus was passaged intracerebrally to 15 groups of baby mice and brains were harvested on the 5th day for dengue 2 and on the 7th day for dengue 3. A 10% suspension in phosphate buffered 4% bovine albumin was made. Of this, 250 ml were stored in bulk at -70°C and the remainder in ampoules for potency, breakthrough neutralization, sterility, and MAP test for murine virus contaminants (done by Microbiological Associates).
- e. Immunization schedule:
Antigen: 20% infected mouse brain in PBS, centrifuged lightly.
day 1 - antigen s.c. (0.5 ml) and i.m. (0.1 ml)
day 3 - antigen s.c. and i.m. as on day 1
Freund's complete adjuvant i.p. (0.1 ml)
day 25- antigen i.p. (0.5 ml)
day 28- antigen i.p. (0.5 ml)
Sarcoma 180 T/G cells i.p.
day 42- harvest ascitic fluid
- f. Control ascitic fluid. Mice were immunized as above except with non-infected baby mouse brain.
- g. CF, HI, and PRNT tests were carried out with established procedures. PRNT tests were in LLC-MK2 cells using a 50% end-point. Sera were inactivated at 56°C for 30 minutes.

Homologous dengue 2 and dengue 3 viruses were used. The Hawaii strain of dengue 1 and the H241 strain of dengue 4 were employed.

III. Results

Production of dengue 2 seed virus reagent was completed. The titer i.c. in baby mice was 9.0 log LD₅₀/ml; 3.7 log LD₅₀ of virus was neutralized by an immune ascitic fluid to the 1bH 10126 strain of dengue 2 in a breakthrough neutralization test. CF test results of mice dying in the breakthrough test indicated no evidence of adventitious agents. Culture for bacterial contaminants was negative as was MAP testing (Table 1).

Production of dengue 3 seed virus reagent was completed. The titer i.c. in baby mice was 7.0 log LD₅₀/ml; 3.6 log LD₅₀ of virus was neutralized by an immune ascitic fluid to the PR-6 strain of dengue 3 in a breakthrough neutralization test. CF results of mice dying in the breakthrough test indicated no evidence of adventitious agents. Culture for bacterial contaminants was negative as was MAP testing (Table 1).

Six lots of dengue 2 ascitic fluid of 5,635 ml total volume were completed. PRNT results are shown in Table 2. Lots 1 and 2 were specific with homologous neutralization 32-fold greater than heterologous. These lots were produced using immunizing antigen prepared from Master seed ahead of time and stored at -70°C prior to inoculation. In an effort to raise homologous titers, subsequent lots were produced using immunizing inocula which were fresh, i.e. not frozen and thawed prior to inoculation. Four-fold or greater increase in titer was thus achieved without sacrificing specificity.

Seven lots of dengue 3 ascitic fluid of 4,215 ml total volume were completed. Representative PRNT results are shown in Table 3. Homologous titers were 4 to 8 fold greater than heterologous. As with the dengue 2 fluids, use of fresh brain suspensions as immunizing inocula gave a 4-fold or greater increase in homologous titer without affecting the specificity.

Control fluids corresponding to the dengue 2 and the dengue 3 immune fluids were produced.

Representative results of CF and HI tests are shown in Tables 4 and 5. The HI test, as expected was broadly cross-reactive. The CF test results were less specific than PRNT.

Minipools were formed using lots 3-6 of the dengue 2 fluids (3,050 ml) and lots 3-7 of the dengue 3 fluids (3,365 ml). Plaque reduction neutralization test results on minipools are shown in Table 6. Comparable test results were obtained in the NIH Pacific Research Laboratory as shown in Table 7.

Cultures of immune and control ascitic fluids for bacteria and mycoplasma were negative.

Table 1

MICROBIOLOGICAL ASSOCIATES • INC



4733 Bethesda Avenue / Bethesda, Maryland 20014 / (301) 654-3400

DIAGNOSTIC SERVICES TEST REPORT

Ref: Our Code #MVS-2036
MVS-2037

TO: Dr. Robert E. Shope
Yale Arbovirus Research Unit
Yale University School of Medicine
60 College Street
New Haven, Connecticut 06510

FROM: Dr. John C. Parker and Dr. Michael Collins

DATE: March 2, 1973

Test: Mouse antibody production test of two arbovirus samples
received December 2, 1972

Specimens: Dengue 2 [NGC] #84850 - MAI Log #MVS-2036
Dengue 3 [H87] #85617 - MAI Log #MVS-2037

Results:

MAI Log No.	Inoculum [dilution]	Viruses found as contaminants by the MAP test											
		PWM	Reo3	Sendai	GDVII	K	Polyoma	MVM	M.Ad.	MHV	LCM	Ectromelia [Vaccinia]	LDH
2036	undiluted	-	-	-	-	-	-	-	-	-	-	-	-
	1:10	-	-	-	-	-	-	-	-	-	-	-	-
2037	undiluted	-	-	-	-	-	-	-	-	-	-	-	-
	1:10	-	-	-	-	-	-	-	-	-	-	-	-

JCP/ps

Table 2

Production of dengue 2 MIAF and PRNT titers

Date	lot #	Approximate Volume in ml	PRNT			
			dengue 1	dengue 2	dengue 3	dengue 4
5/30/72 to 6/12/72	1 [†]	1,505	<10 [*]	<u>320</u>	<10	<10
8/3-13/72	2 [†]	1,080	20	<u>720</u>	<10	<10
12/22/72	3	900	30	<u>4,100</u>	35	10
12/30/72	4	750	50	<u>2,000</u>	30	10
2/27/73	5	750		<u>15,000</u>		
3/2/73	6	650		<u>4,500</u>		

* 50% plaque reduction in LLC-MK2 cells.

† Lots 1 and 2 were not included in the final pool because of low homologous titers.

Table 3

Production of dengue 3 MIAF and PRNT titers

Date	lot #	Approximate Volume in ml	PRNT			
			dengue 1	dengue 2	dengue 3	dengue 4
8/21/72	1	350	80*	110	<u>620</u>	28
8/28/72	2	500	500	230	<u>3000</u>	80
12/22/72	3	600	740	300	<u>1500</u>	65
3/27/73	5	750		240	<u>1200</u>	

* 50% plaque reduction in LLC-MK2 cells.

Table 4

**Results of CF tests with Dengue 2 and Dengue 3
Ascitic Fluids**

Ascitic Fluid	Dengue 1	Dengue 2	Dengue 3	Dengue 4	Normal
D-2 lot 1A	16*	32	16	16	0
1B	64	128	32	32	0
1C	16	32	8	16	0
2A	16	64	16	16	0
2B	128	>128	>128	>128	0
2C	64	128	128	64	0
5	128	128	1024	256	0
6	128	64	512	128	0
D-3 lot 1	128	32	>128	>128	0
2	32	16	>128	32	0
5	ND	ND	256	ND	0
6	ND	ND	256	ND	0
7	ND	ND	256	ND	0

* Reciprocal of Ascitic fluid titer; ND=Not Done.

Table 5

Results of HI tests with Dengue 2 and Dengue 3

Ascitic fluids

Ascitic Fluid	Antigens (8-16 units)				JE
	Dengue 1	Dengue 2	Dengue 3	Dengue 4	
D-2 lot 1A	40	160	40	40	20
1B	40	320	40	40	40
1C	40	320	80	40	40
2A	40	160	80	80	40
2B	40	320	80	80	80
2C	40	320	160	160	40
D-3 lot 1	160	320	2560	320	80
2	640	640	5120	1280	320

Table 6

Plaque Reduction Neutralization Titers with Minipools

Ascitic fluids	<u>Viruses</u>			
	D1	D2	D3	D4
Dengue 2	40	<u>5120</u>	25	30
Dengue 3	80	180	<u>1200</u>	40



Table 7

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
~~UNITED STATES DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE~~

PACIFIC RESEARCH SECTION
P. O. BOX 1680
HONOLULU, HAWAII 96806

December 10, 1973

Dr. Robert E. Shope
Director, Yale Arbovirus
Research Unit
Department of Epidemiology
& Public Health
Yale University School of Medicine
60 College St.
New Haven, Conn. 06510

Dear Bob:

We obtained the following results in our 90% plaque reduction
neutralization test with the mouse ascitic fluid you sent some time
ago.

Serum	V i r u s			
	D1	D2	D3	D4
D2	40	640	80	40
D3	160	320	2560	40

If you have any questions please let me know.

Sincerely,

Leon Rosen, M.D.
Head, Pacific Research Section
National Institute of Allergy
and Infectious Diseases

Table 8

Results of HI tests with dengue 2 and
dengue 3 ascitic fluids

Antigens	(units)	Antibody	
		dengue 2	dengue 3
Powasson	(8)	40	160
Modoc	(8)	40	160
MML	(16)	<10	<10
Cowbone Ridge	(16)	40	80
Rio Bravo	(16)	160	320
Ilheus	(16)	80	320
St. Louis	(8)	40	80
dengue 2	(16)	160	320
dengue 3	(8)	160	640
yellow fever	(4)	320	160
Japanese encephalitis	(8)	640	640
West Nile	(8)	160	640

Table 9

Results of CF Tests with Dengue 2 and Dengue 3
Minipool Ascitic Fluids

Antigens	<u>Antibody</u>			
	dengue 2	dengue 2 control	dengue 3	dengue 3 control
dengue 1	128/64 [*]	0/0	128/64	0/0
dengue 2	512/64	0/0	128/64	0/0
dengue 3	128/64	0/0	512/256	0/0
dengue 4	128/64	0/0	128/64	0/0

^{*}Reciprocal of antibody/reciprocal of antigen titers.